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BIODEGRADATION AND DECOLOURIZATION OF AZO DYES USING MARINE BACTERIA

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ABSTRACT

The azo aromatic is the most widespread dye class in the industry. They may have one or more azo (N=N) groups. The most commercially important are mono-azo dyes and di-azo dyes, tri-azo dyes, whereas poly azo are much less important. The disadvantage of these dyes are not easily decomposed by aerobic bacteria, but are degraded by anaerobic bacteria to aromatic amines like naphthylamines, chloro aniline. The dyes when they are exposed to the environment comprises a little percentage of water pollution, but in turn it is a indigenous environmental pollution and a public health concern. Two dyes such as Congo red and Erichrome Black T were used for the studies. Marine water sample was collected from Arabian Sea (Calicut) and Bay of Bengal (Ramanathapuram) and the laccase producing bacterial colonies were isolated.

The marine bacterial laccases was characterized at different pH and temperature. Berge's Manual of Systemic Bacteriology has been used for describing the Bacteria. Then the isolates are observed for the decolourization of dyes under optimized conditions. The confirmation of dye decolourization was done through the spectral analysis at the wavelength range of 260 to760 nm at regular intervals of 5 nm.

KEYWORDS: Water Pollutant, Industrial Effluent, Halophilic Bacteria, Azo Dyes & Bioremediation

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Original Article

INTRODUCTION

Environmental pollution is one of the major problems of the modern world. Industrialization is necessary to satisfy the needs of the world's growing population but it threatens life on earth by polluting the environment. The problem of environmental pollution is increasing day by day due to the release of recalcitrant substances like pesticides, dyes, polymers, etc and heavy metals. Textile dyes pose environmental hazards because of colour and toxicity. Synthetic dyes are coloring retailers particularly used in textile industries which generate a large quantity of waste water inside the procedure of dyeing. While colored natural compounds generally impart handiest a minor fraction of the natural load to wastewater, their coloration renders them aesthetically unacceptable.

Synthetic dyes are considerably used in textile dyeing, paper printing, colour photography, pharmaceutical, meals, cosmetics and different industries. About 10-15% of the dyes are launched into the environment. Main training of artificial dyes used is azo dyes, anthraquionone and triphenylmethane. In addition to their visual effect and damaging effect in terms of chemical oxygen call for (COD), many synthetic dyes show their toxic, carcinogenic and genotoxic consequences.

Azo Dyes

A dye can generally be defined as a coloured substance that has an affinity to the substrate to which it's far being applied. The dye is typically implemented in an aqueous answer, and may require a mordant to improve the fastness of the dye at the fiber. Both dyes and pigments appear like coloured due to the fact they soak up some wavelengths of mild preferentially. In comparison with a dye, a pigment usually is insoluble, and has no affinity for the substrate.

Azo dyes are the biggest synthetic chemical substances which are utilized in numerous industries. Most of the azo dyes are both inert and non-poisonous, but they emerge as poisonous, mutagenic and carcinogenic upon biotransformation. Azo dyes are the main chemical class of dyes with the greatest variety of colours, therefore they have been extensively used by the industry, these dyes are characterized by one or more azo linkages (R_1-N=N-R_2) and aromatic structures, Degradation of dyes, especially azo dyes which comprise about 70% of all dyes used, is difficult due to their complex structure and synthetic nature.

Chromophores

It is functional groups which are unsaturated and they cause a compound to become coloured.

Examples of chromophores are –N=N-, -C=C-, -C=N- and -C=O.

Auxochromes

It's far a corporations that does no longer impart color to the compound however growth the coloration of the compound. Useful corporations which includes hydroxyl (–OH), amino (-NH2), nitro (-NO2), alkyl (-R), —OH, —OR, —NH2, —NHR, —NR2, —SH are examples for auxochrome.

General Properties of the Azo Dye

- Impart colour to water bodies even if present in small amount
- Reduces mild penetration and photosynthesis.
- Carcinogenic or mutagenic
- Azo dyes are greater toxic as they have an effect on microbes thereby affecting biological degradation treatment.
- Dyes will increase BOD of effluent thereby affecting aquatic existence
- Toxic to fish & microbial organisms
- The discharge of heavy metals into aquatic ecosystems ends in boom in alkalinity of water
- The turbidity and colour in conjunction with oil and scum create an unsightly appearance.
- The mineral materials, by and large sodium salts increase salinity of the water.

Textile Dye Treatment

There are various chemical, physical and organic shade removal techniques paintings either by way of concentrating the color into sludge, solid helps, or by the complete destruction of the dye molecule. It’s far predicted that decolourization structures involving destruction technologies will succeed, because the switch of pollutants from one part of the surroundings to some other is averted. currently, the fundamental methods of fabric dye treatment involve physical

and/or chemical procedure as membrane filtration, coagulation, flocculation, precipitation, floatation, adsorption, ion pair extraction,

Ultrasonic mineralization, electrolysis, chemical discount and advanced chemical. The superior oxidation, photograph catalytic oxidation and moist-air oxidation. Because of the excessive value and disposal troubles, maximum of the chemical and bodily methods for treating dye wastewater were not extensively implemented in the fabric industries. There's additionally the possibility that secondary pollutants problem will arise due to excessive chemical use. Organic treatments have been conventionally applied present positive drawbacks.

Microbial groups are of primary importance in bioremediation of metal contaminated soil and water, because microbes modify steel chemistry and mobility through reduction, accumulation, mobilization and immobilization. organic methods the usage of various microbes like micro organism, fungi and algae of dye elimination will be a possible alternative as a low-cost and eco-friendly decentralized effluent remedy system for small-scale industries. The ability of bacteria to metabolize azo dyes has been investigated with the aid of a number or research groups. Underneath aerobic conditions azo dyes are not readily metabolized, even though the ability of micro organism with specialized reducing enzymes to aerobically degrade sure azo dyes changed into stated. In assessment, underneath anaerobic conditions many micro organism reduce azo dyes by using the hobby of unspecific, soluble, cytoplasmic reductase, called azo reductases. The anaerobic reduction degrades the azo dyes which might be transformed into aromatic amines (Yaropolov et al., 1994) which can be poisonous, mutagenic and in all likelihood carcinogenic to mammalians. Consequently, to reap entire degradation of azo dyes, another level that involves cardio biodegradation of the produced aromatic amines is essential. Bacterial biodegradation of non-azo dyes has most effective these days been studied.

Laccases

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) catalyze the oxidation of various aromatic, specifically phenolic substrates (e.g. hydroquinone, guaiacol, 2, 6-dimethoxyphenol or phenylene diamine), coupled to the reduction of molecular oxygen to water. Laccases as well as ascorbate oxidases (EC 1.10.3.3) and ceruloplasmins/ ferroxidase (EC 1.16.3.1) typically include several copper atoms within the catalytic centre. Therefore, they belong to the enzyme super family of multicopper oxidases, which is a extensively dispensed protein own family among pro- and eukaryotes.

Laccases are involved within the biodegradation of many continual environmental pollution like azo dyes, polycyclic fragrant hydrocarbons, endocrine disruptors or polycyclic musk fragrances.

Structure of Laccase

The catalytic hobby of fungal laccases requires at least 4 copper atoms in keeping with energetic protein. For this reason, laccases encompass 4 regions, which bind the copper atoms of the energetic centre. Those copper binding areas (cbr) are strongly conserved in all laccases and had been used for the design of degenerate primers to research and perceive laccase genes of numerous species or in environmental samples.

Application of Laccases

The programs include the detoxification of business effluents, commonly from the paper and pulp, textile and petrochemical industries. They’re used as a tool for scientific diagnostics and as a bioremediation agent to smooth up

herbicides, insecticides and certain explosives in soil. Laccases are also used as cleansing sellers for sure water purification structures. They are used as catalysts for the manufacture of anti-cancer tablets and whilst ingredients in cosmetics. Similarly, their potential to get rid of xenobiotic substances and convey polymeric merchandise makes them a beneficial device for bioremediation functions.

OBJECTIVES

- To screen laccase secreting bacteria from marine water.
- To characterize the laccase enzyme activity.
- To decolorize and biodegrade the azo dyes in simulated and real textile wastewater using marine bacteria.

REVIEW OF LITERATURE

Distribution of Laccases in Bacteria

The first report of prokaryotic laccase is from the rizospheric bacterium *Azosprillium lipoferum (*Givaudan *et al., 1993),* where laccase occurs as a multimeric enzyme composed of a catalytic subunit and one or two large chains. Laccase has also been reported from a marine melanogenic bacterium *marinosomonas mediterranea* producing two different polyphenol oxidases (Solano *et al.,* 1997). A laccase like enzyme activity was also found in spores of B*acillus sphaericus* (Claus and Filip, 1997**).**

- **A Typical Laccase Reaction where a Diphenol (Hydroquionone) undergoes a One-Electron Oxidation to form an Oxygen-Centred Free Radical. Quionone and Other Radicals under Goes Polymerization. (Thurston, 1994)**

For catalyzing the oxidation of non-phenolic substrates, laccase calls for the presence of a mediator within the medium. A mediator is a small molecule that behaves like an 'electron travel' among laccase and substrate and those small molecular-mass compounds are transformed into strong radicals by means of enzymatic oxidation. They act as redox mediators and oxidize different compounds that, in precept, are not substrates of laccase. From the outline of the primary laccase mediator, 2,2¢-azinobis (three- ethylbenzothiazoline-6-sulphonic acid) (ABTS) to more latest use of the—NOH-kind synthetic mediator, which includes 1-hydroxybenzotriazole (HBT), violuric acid (VLA) and N-hydroxyacetanilide (NHA), a huge range of studies were produced on the mechanisms of oxidation of non-phenolic substrates (Baiocco et al. 2003). Using obviously going on mediators could gift environmental and financial advantage (Camarero et al. 2005). The enzyme possesses fantastic biotechnological potential due to its wide response talents in addition to large substrate specificity. Promising applications include biosensors for drug analysis and phenols in tea (Ghindilis *et al.* 1992; Peter and Wollenberger 1997), polymer synthesis (Huttermann *et al.* 2001), textile-dye bleaching (Claus *et al.* 2002), bioremediation (Murugesan 2003; Wesenberg *et al.* 2003), fungicidals (Spillman 2003) pulp bleaching (Palonen and Viikari 2004), clarification of juices and wines (Ygshinwa 2004).

Screening of Bacteria Secreting Laccase

Microbes that produce laccases have been screened on strong media containing coloured indicator compounds that enable the visualization of laccase manufacturing (Nishida et al; De jonget al., 1992; Barbosa et al). The conventional screening reagants together with tannic acid and gallic acids (Harkin and Obst, 1973) have now a days on the whole been replaced by way of synthetic phenolic reagents inclusive of guaiacol and syringaldazine (Nishida et al 1992; De jong et al.,

1992).With guaiacola wonderful reaction is indicated by way of the formation of a pink – brown halo (Nishida et al., 1988). Discovery of novel laccases with exclusive substrate specificities and improved stabilities is important for commercial programs. Microbes that produce laccases had been screened for both on stable media containing coloured indicator compounds that allow the visible detection of laccase manufacturing (Nishida et al., 1988; De Jong et al., 1992; Barbosa et al., 1996) or with liquid cultivations monitored with enzyme hobby measurements (Szklarz et al. 1989; Pela´ez et al. 1995; Luterek et al. 1997). the usage of coloured indicators is usually simpler as no pattern dealing with and measurement is required. As laccases oxidize various styles of substrates, numerous different compounds were used as signs for laccase manufacturing. The conventional screening reagents tannic and gallic acid (Harkin and Obst 1973) have in recent times ordinarily been replaced with synthetic phenolic reagents, inclusive of guaiacol and syringaldazine (Nishida et al.1988; De Jong et al. 1992) or with Remazol remarkable Blue R (RBBR) and Poly R-478 (Barbosa et al. 1996; D'Souza et al. 1999; Raghukumar et al. 1999).RBBR and Poly R-478 are decolourized through lignin-degrading fungi (Gold et al. 1988; Barbosa et al. 1996), and the manufacturing of ligninolytic enzymes is discovered as a colourless halo round microbial boom. With guaiacol a wonderful reaction is indicated via the formation of a reddish-brown halo (Nishida et al. 1988), while with tannic and gallic acid the nice response is a dark-brown colored area (Harkin and Obst 1973).Laccase when uses syringaldazine as substrate, then the initial product is a free radical. The quionone formed by a second one-electron oxidation is deep purple in colour and not apparently prone to polymerization.

- **Action of laccase on Syringaldazine (Thurston, 1994)**

DYE

A dye can be defined as a coloured substance that has an affinity towards the substrate in which it has been applied. The dye is normally applied in an aqueous answer, and might require a mordant to enhance the fastness of the dye at the fibre (Zollinger, 2002).In other words, dye is defined as a substance, generally natural, that is designed to be absorbed or adsorbed via made to react with, or deposited inside a substrate with a purpose to impart coloration to the substrate with a few diploma of permanence.

Dyes are used in fabric industry, leather tanning industry, paper production, meals enterprise, agricultural research, light-harvesting arrays, photoelectrochemical cells, hair colouring and cosmetics. Moreover those compounds were hired for the control of the efficacy of sewage and wastewater treatment for the dedication of precise surface area of activated sludge and for ground water tracing (Forgacs et al., 2004). Since large quantities of water used for dyeing the fabrics, textile industry is one of the major polluting industry in India.

AZO DYES

The azo aromatic one is the most massive dye elegance in the industry. They may have one or extra azo (N=N) agencies. Azo dyes with one azo institution are known as mono-azo dyes, with azo agencies, di-azo dyes, observed by tri-azo azo dyes with more than three azo linkages are special polyazo dyes. (Pierce, 1994). The maximum commercially important are mono-azo dyes and di-azo dyes, tri-azo dyes, while polyazo are much less critical. The primary drawback of this magnificence of dyes is that they may be not without problems degraded by means of aerobic bacteria, and with the action of anaerobic or microaerobic reductive bacteria.

They're representing almost 70% of the textile dyestuffs produced. They may be smooth to synthesize, have low price, are strong, can be used to color numerous materials and permit a notable variety of hues and sun shades. They have

got in their molecule one or extra azo businesses. They may be acquired from the coupling of diazonium salts with aromatic amines, phenols, naphthols or aliphatic enols. The diazonium salts received from the reaction of sodium nitrite with an amine answer with a mineral acid, ideally HCl (Zollinger et al.,2002).The different types of dyes include Acid dye, Reactive dye, Direct dyes, Basic dyes, Disperse dyes, Pigments dyes, Vat dyes, Azoic and Ingrain dyes, Sulphur dyes.(Neill *et al*., 1999 and Rocha Gomes,2001).Techniques for the elimination of BOD from most effluents are fairly properly hooked up. Dyes, but, are greater hard to treat due to their artificial origin and complicated aromatic molecular structures (Banat et al., 1996).there may be a splendid environmental concern approximately the fate of these dyes, and their dangerous metabolites particularly on reactive dyeing of cellulosic fibers, wherein large amounts of unbound dye are discharged inside the effluent (Pierce, 1994). They are able to form toxic and/or mutagenic compounds including aromatic amines like naphthylamines, chloro aniline and many others… (Chung et al., 1992; Wong et al., 1996)

MATERIALS AND METHODOLOGY

Screening of Microbes for Laccase Enzyme

- Marine water sample was collected from Arabian sea (Calicut) and Bay of Bengal (Ramanathapuram).
- The sample was serial diluted from 10-1 to 10-6 and plated on the MH medium (Refer appendix 1).The isolated colonies were plated on the MH agar medium incorporated with 0.02% guaiacol and incubated for 72 hrs at 37□C.
- Identification of bacteria was done by the method described in Bergey's Manual of Systemic Bacteriology.

Determination of Optimum Wavelength for Reactive Dyes

- The maximum absorbance differs with dyes. It differs for each dye.
- 100ml of Mueller and Hinton medium (Refer appendix 1) was prepared and 2ml of respective dyes were added from stock (Refer appendix 2).
- The absorbance was measured spectrophometrically at an increment of 5 nm wavelength ranging from 260nm to 760 nm.
- The wavelength for which maximum absorbance obtained was used to measure the dye decolourization in subsequent studies.

Test for Dye Decolourization Efficiency

- The dye decolourization medium (Refer appendix 3) was prepared.
- 100 µl of overnight grown culture was used as inoculum to inoculate the above media and the flasks were incubated for 24 hrs at 37°C.
- The results were observed.

  Dye removal (%) is calculated as

  $$\textbf{Dye removal (\%)} = \frac{\textbf{Initial absorbance – Final absorbance}}{\textbf{Initial Absorbance}} \times 100$$

Study of Growth Profile

- The MH medium (Refer appendix 2) was prepared and sterilized
- Isolated bacterial colony was inoculated on the above media.
- The absorbance was taken at different time interval (0 – 96 hrs) at 660 nm.

Laccase Assay

For Substrate Guaiacol

- The reaction mixture consists of 3ml buffer (pH 8, 0.1M phosphate), 1ml guaiacol, and 1ml of enzyme(supernatant of the culture)
- Incubate for 5 minutes.
- Measure the OD on 450nm.
- Enzyme activity is defined as amount of enzyme causing 1µM substrate conversion per second.

For Substrate O-Dianisidine

- The reaction mixture consists of 600µl buffer (pH 8, 0.1M phosphate), 100µl o-dianisidine, and 100µl of enzyme(supernatant of the culture)
- Incubate for 10 mins at 55□C.
- Measure the OD on 450nm

For Substrate ABTS

- The reaction mixture consists of 940µl of buffer (pH 8, 0.1M phosphate), 10µl of ABTS, 50µl of enzyme(supernatant of the culture)
- Incubate for 15 mins at 37□C.
- Measure the OD on 420nm.
- Enzyme activity is defined as amount of enzyme causing 1µM substrate per minute.

  The same procedure is performed for **different pH** and **temperature**.

Laccase Activity on Different Carbon Source

- Different carbon sources was used in MH medium (Refer appendix 2) instead of glucose.
- Incubated for 24 hrs and the activity were measured at different time intervals.
- Growth profile was also studied.

Laccase Activity on Different Nitrogen Source

- Different nitrogen sources was used in MH medium (Refer appendix 2) instead of glucose.
- Incubated for 24 hrs and the activity were measured at different time intervals.

- Growth profile was also studied.

Spectral Analysis of Degraded Dye Samples

- The degraded sample was extracted using the solvent Ethyl acetate.
- Then it was subjected to spectral analysis over the range of 260 - 760nm.
- On comparing the results with authentic dye gives the conformation of degradation.

PURIFICATION OF LACCASE

- The overnight culture in MH medium (refer appendix 2) was precipitated with acetone(1:1).
- The precipitated sample was centrifuged.
- The supernatant was collected and dialysis was carried out

RESULTS AND DISCUSSIONS

Screening of Microbes for Laccase Enzyme

Petri plates inoculated from serially diluted test tube has shown individual colonies after 2 days of incubation.

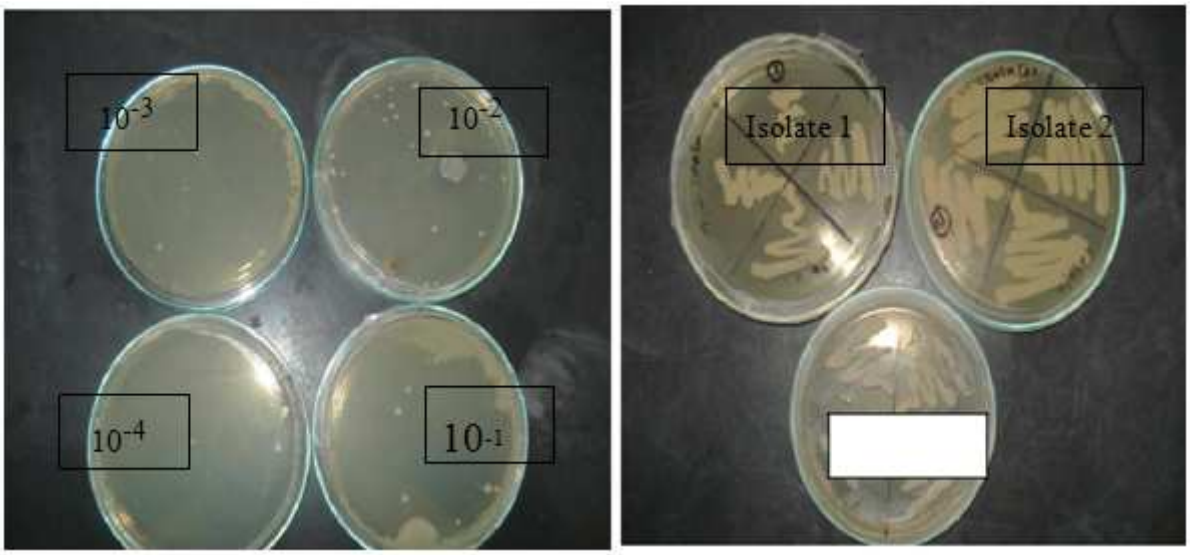

Figure 1: Screening of Microbes for Laccase Enzyme

Addition of 0.02% of guaiacol to the MH media (Refer Appendix 2).The grown culture plates had developed red colour halo in isolate 2.

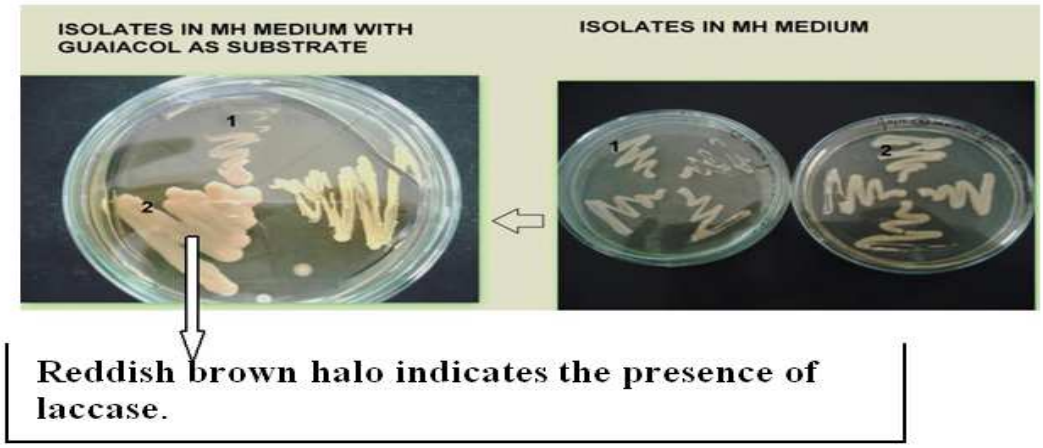

Figure 2: Presence of Laccase

REDDISH BROWN HALO INDICATES THE PRESENCE OF LACCASE

Confirmation of Laccase

Single colony was inoculated in MH medium (Refer Appendix 2). After 24hrs.02% 0f guaiacol was poured on the plate.

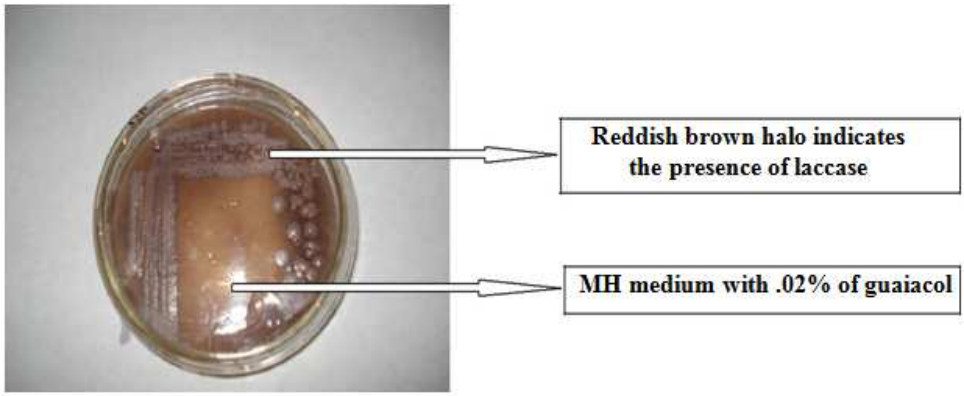

Figure 3: Confirmation of Laccase

Biochemical Tests for the Isolates

Table 1

Tests	Isolate	Positive/Negative
Gram's Staining	2	Positive
Catalase test	2	Positive
Oxidase test	2	Positive
Starch-Iodine test	2	Positive

From the above biochemical tests we can conclude that,

Isolate 2 – *Bacillus* sp.1

Decolourization of Dye

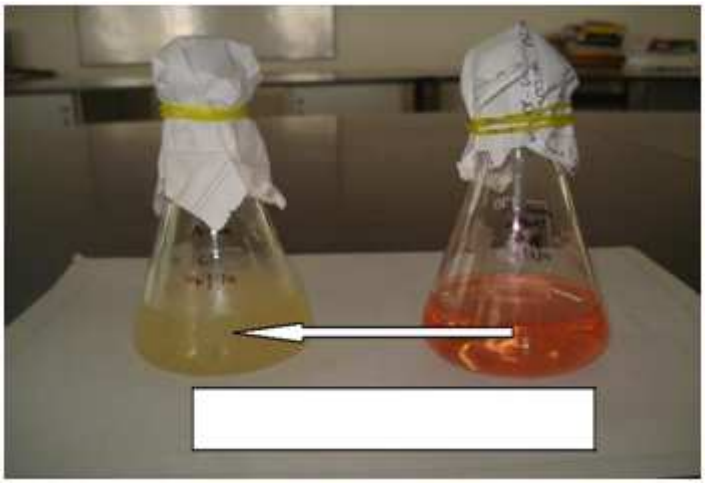

Figure 4: Decolourization of Congo Red

Decolourization after 96hrs

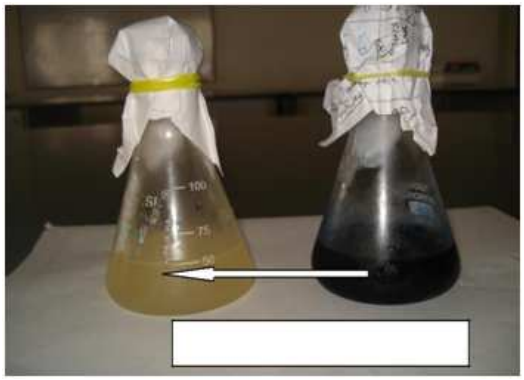

Figure 5: Decolourization of Erichrome. Black. T

Decolourization after 96hrs

From figure 4 and 5, it was observed that dye was decolourized completely after 96hrs

CONCLUSIONS

- The bacterial colonies were isolated from marine water and followed by the method described in **Bergey's Manual of Systemic Bacteriology,** the isolate was identified as *bacillus sp*.
- They were screened for laccase production using guaiacol as substrate.
- The decolorization and biodegradation the azo dyes in simulated and real textile wastewater using above isolates was carried out. It was observed that the dye was completely decolourized after 96 hrs and % dye degradation was analyzed for Congo Red and Erichrome Black. T.
- The degradation of dyes were confirmed through Spectral Analysis over the wavelength range of 260 to760 nm at regular intervals of 5 nm.
- The laccase enzyme activity was characterized at different pH, Temperatures, Carbon and Nitrogen source.

Optimized conditions for the laccase activity are,

pH	**8**
Temperature	**30□C**
Carbon source	**Fructose**
Nitrogen source	**Beef extract**

- The growth profile of the organism was observed for different Carbon and Nitrogensource in the MH medium

REFERENCES

1. *Adedayo O., Javadpour S., Taylor C., Anderson W.A., Moo-Young, M. (2004). Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. World Journal of Microbiology and Biotechnology 20, 545–550.*
2. *Alexandre G., Zhulin LB. (2010) Laccases are widespread in bacteria. TrendsBiotechnol18:41–42*
3. *An S.Y., Min S.K., Cha I.H, Choi Y.L, Cho Y.S. and Kim C.H, (2002). Decolorization of triphenylmethane and azo dyes by Citrobacter sp. Biotechnol Lett; 24:1037–40.*
4. *Baiocco P., Barreca A.M., Fabbrini M., Galli C. and Gentili P. (2003). Promoting laccase activity towards non-phenolic substrates: a mechanistic investigation with some laccase-mediator systems.Org Biomol Chem 1:191–197*
5. *BanatI.M.,NigamP.,Singh D. and Marchant R. (2008). Microbial decolourization of textile-dye-containing effluents, BioresTechol, 58:217-227.*
6. *Blumel S., Knackmuss H.J., Stolz A. (2002). Molecular cloning and characterization of the gene coding for the aerobic azoreductase from XenophilusazovoransKF46F.Appl Environ Microbiol.68:3948–55.*
7. *Brown N.L, Barrett S.R, Camakaris J., Lee B.T., Rouch D.A. (1995). Molecular gene and transport analysis of the copper resistance determinant (pco) from Escherichia coli plasmid pRJ 1004.MolMicrobiol17:1153–1166*
8. *Camarero S., Ibarra D., Martinez M.J., Martinez A.T. (2005). Lignin derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. Appl Environ Microbiol. 71:1775–1784*
9. *Cervantes F.J., Duong-Dac T., Ivanova A.E., Roest K., Akkermans A.D.L., Lettinga G., et al. (2010). Selective enrichment of Geobacter sulfurreducens from anaerobic granular sludge with quinones as terminal electron acceptors. BiotechnolLett;25:39–45.*
10. *Chang C.N., Lin J.G., Chao A.C., Liu C.S. (1996). Modified Nernst model for on-line control of the chemical oxidation decoloring process. Water SciTechnol.;34:151–7.*
11. *Chang J.S., Kuo T.S., Chao Y.P., Ho J.Y., Lin P.J. (2000). Azo dye decolorization with a mutant Escherichia coli strain. BiotechnolLett.;22:807– 12.*
12. *Chang J.S., Lin C.Y. (2001). Decolorization kinetics of a recombinant Escherichia coli strain harboring azo-dye-decolorizing determinants from Rhodococcussp. BiotechnolLett.; 23:631–6.*
13. *Chen K.C., Wu J.Y., Huang C.C., Liang Y.M., Hwang S.C.J. (2003). Decolorization of azo dye using PVA-immobilized microorganisms. J.Biotechnol.; 101:241–52.*
14. *Claus H. (2003) Laccases and their occurrence in prokayotes. Arch. Microbiol 179:145–150*

15. *Claus H., Faber G., Konig H. (2002). Redox-mediated decolorization of synthetic dyes by fungal laccases. ApplMicrobiolBiotechnol.;59:672–678.*

16. *Claus H., Filip Z. (1997). The evidence of a laccase-like activity in a Bacillus sphaericuss train. Microbiol Res.;152:209–215*

17. *Driks A. (2004) The Bacillus subtilis spore coat. Phytopathology; 94:1249–1251*

18. *Faure D., Bouillant M., Bally R. (1994). Comparative study of substrates and inhibitors of Azospirillum lipoferum and Pyriculariaoryzae laccases. Appl Environ Microbiol.; 61:1144–1146*

19. *Faure D., Bouillant M., Bally R. (1994). Isolation of Azospirillum lipoferum 4T Tn5 mutants affected in melanization and laccase activity. Appl Environ Microbiol.; 60:3413–3415*

20. *Forgacs E.,Cserhati T. and Oros G. (2004). Removal of synthetic dyes from waste water: a review. Environ Int.; 30:953-71.*

21. *Ghindilis A.L, Gavrilova V.P., Yaropolov A.I. (1992) Laccase based biosensor for determination of polyphenols: determination of catechols in tea. BiosensBioelectron7:127–131*

22. *Givaudan A., Effosse A., Faure D., Potier P., Bouillant ML, Bally R (1993) Polyphenol oxidase in Azospirillum lipoferum isolated from rice rhizosphere : evidence for laccase activity in nonmotile strains of zospirillum lipoferum. FEMS Microbiol Lett 108:205–210*

23. *Goncalves MST, Oliveria-Cmpos AMF, PntoEM, Plasencia PMS and Queiroz MJ (1999),Photochemical treatment of solutions of azodyes containing TiO2,Chemosphere 39:781-786*

24. *Hough MA, Hall JF, Kanbi LD, Hasnain SS (2001) Structure of the M148Q mutant of rusticyanin at 1.5A: a model for the copper site of stellacyanin. Acta Crystallogr 57:355–360*

25. *Huttermann A, Mai C, Kharazipour A (2001) Modification of lignin for the production of new compounded materials. ApplMicrobiolBiotechnol 55:387– 394*

26. *Itoh K, Kitade Y, Kobayashi S, Nakanishi M, Yatome C.(1998) Demethylation of acridine orange by Arthrobacter globiformis. Bull Environ Contam Toxicol 60:781– 5.*

27. *Itoh K, Kitade Y, Nakanishi M, Yatome C.(2002) Decolorization of methyl red by a mixed culture of Bacillus sp. and Pseudomonas stutzeri. J Environ Sci Health Part A, Environ SciEng Toxic Hazard Substance Control A;37:415–21.*

28. *Kasper, H.F., Wuhrmann, K. (1978). Kinetic parameters and relative turnover of some important catabolic reactions in digesting sludge. Applied and Environmental Microbiology 36, 1.*

29. *Kawai S, Umezawa T, Shimada M, Higushi T (1988) Aromatic ring cleavage of 4,6-di(tert-butyl)guaiacol, a phenolic lignin model compound, by laccase of coriolus versicolor. FEBS Lett 236:309–311*

30. *Koneva ND, Kruglov YV.(2001). The dynamics of the size and structure of the soil bacterial complex in the presence of azobenzene. Microbiology; 70:480– 3.*

31. *Laszlo JA. (2000).Regeneration of azo-dye-saturated cellulosic anion exchange resin by Burkholderia cepacia anaerobic dye reduction. Environ SciTechnol; 34:167– 72.*

32. *Martins LO, Soares CM, Pereira MM, Teixeira M, Costa T,Jones GH, Henriques AO (2002) Molecular and biochemical characterization of a highly stable*